SPECIFICATION

FOR

A COMPOUND AND METHOD OF TREATMENT FOR FUNGAL PATHOLOGIES OF THE ORAL CAVITY

BACKGROUND OF THE INVENTION

This invention relates to the field of pathologies of the oral cavity caused by fungi.

One of the most common and stubborn infections of the mouth and throat is Candidiasis. Thrush or acute candidiasis is caused by extensive candidal invasion of the oral mucosal epithelium. Thrush presents as creamy, yellow tufts which can be readily wiped off with a swab to expose a red and inflamed area of epithelium. The cottony tufts are the result of extensive infiltration of candidal hyphae into the mucosal epithelium.

Although thrush is most common in infants, it also occurs in adults that are immunocompromised, undergoing broad spectrum antibiotic treatment, undergoing corticosteroid treatment, diabetics and anemics. In adult patients, particularly immunosuppresed patients, thrush is treated with large amounts of azole compounds such as miconazole, itraconazole, econazole, ketoconazole or fluconazole. Among thrush patients not already undergoing broad-spectrum antibiotic treatment, broad-spectrum antibiotics are frequently prescribed in combination with fungicide therapy to avoid or treat any secondary bacterial infections. Infants are usually treated with fungicidal suspensions if the infection does not spontaneously clear.

Chronic candidiasis is both far more common and far more difficult to treat than thrush. Candidiasis is characterized by less extensive innervation of the epithelium by the Candidal hyphae than thrush.

Candidal proliferation on denture surfaces is a common, chronic, but minor form of candidiasis. Inflammation of the epithelium under dentures, denture stomatitis, may be caused by the proliferation of *C. albicans* in the interface between the denture-bearing mucous membrane and the denture surface. The lower denture-bearing area is typically freely exposed to saliva, and consequently denture stomatitis is rarely seen in this site. However, a close-fitting upper denture creates a microenvironment cut-off from any protective effects of saliva. When *C. albicans* proliferates under a denture, it is held in prolonged contact with the mucous membrane and presumably acts as an irritant.

Denture stomatitis is considered a common cause of inflammation of the epithelium near the corners of the mouth, angular stomatitis, among ambulatory patients. Typical treatment requires vigorous use of topical oral nystatin or amphotericin B to resolve denture stomatitis and associated angular stomatitis. During treatment, the dentures must be worn as little as possible to allow the drug to the reach affected area, thus, inconveniencing suffering denture wearers.

Candidal leukoplakia is a less common type of chronic candidiasis, appearing as a thick keratizined lesion on or about the tongue. Microscopically, a candidal leukoplia plaque consists of a thick layer parakeratinized epithelium invaded by candidal hyphae. These keratinized plaques of chronic candidiasis are tough, adherent, and often irregular in thickness, persistent, and therefore, unlike the soft friable plaques of thrush. Common sites are the commissures of the tongue and cheeks. Treatment of candidal leuloplakia infections are difficult since the intracellular growth of the candida makes it less accessible to antifungal drugs. Absent

treatment, these leukoplakias often cover large areas of the mouth and tongue, making eating painful and causing significant social anxiety among the afflicted.

A number of rare forms of chronic mucocutaneous candidiasis appear associated with various immune disorders. Familial chronic mucocutaneous candiasis is a rare, recessive, immune disorder which confers a tendency to develop chronic, leukoplakia-like plaques. Diffuse chronic mucocutaneous candidiasis is associated with susceptibility to bacterial infections, particularly of the respiratory tract, and other fungal infections. Its lesions may extend down the pharynx or larynx and when affecting the mouth and lips, can be severely disfiguring. Candida endocrinopathy syndrome appears to be transmitted as an autosomal recessive gene. The candidal infections tend to be mild and dermal lesions are rare. The main features are the associated endocrine deficiencies. Most common is hypoparathyroidism, but hypoadrenalism and almost any other type of endocrine deficiency can develop.

Treatment for all these candidal immune disorders are difficult. If the immunological defect can be identified, as is sometimes the case with diffuse chronic mucocutaneous candidiasis, treatment may be possible, but generally treatments are ineffective because they fail to address the underlying immunological disorder that allows the candida to flourish.

The foregoing discussion indicates that an effective fungicide against a wide range of candidal pathologies useful in immuno-compromised patients, children, and the elderly that can either be topically applied or systemically applied to treat the chronic candidal infections like candidal leukoplakia that is resistant to topical treatment would be a significant advance to the art. Such a fungicidal should preferably have low toxicity and even more preferably possess anti-inflammatory properties. Lastly, the fungicidal should also have minimal cross reactivity

with other drugs so that it may be simultaneously prescribed with the complicated treatment regimens of the elderly and chronically ill—two groups likely to suffer candidiasis.

SUMMARY OF THE INVENTION

The invention includes a composition and method of treatment of fungal pathologies of the oral cavity or fungal growth on the surface of dentures. In a preferred embodiment of the invention a therapeutically effective amount of one or more peptides selected from the group of peptides with a C-terminal sequence consisting of KPV, HFRWGKPV, and SYSMEHFRWGKPV used in combination with a therapeutically effective amount of a fungicide selected from the group consisting of: itraconazole, econazole, ketoconazole, miconazole and fluconazole and dissolved into a carrier. More preferably still each peptide has the primary sequence of KPV or VPK-Ac-CC-Ac-KPV (Ac=Acetyl group). Pharmacologically effective concentrations may be as low as 10^{-12}M but may be as high 10^{-4} M. Pharmacologically effective concentrations of these peptides may be incorporated into commercial formulations of creams, gels, mouthwashes, toothpastes, tablets, or atomized sprays.

In another preferred embodiment of the invention these peptides are topically or systemically applied to treat a candida infection of the oral cavity. In yet another embodiment of the invention these peptides are used in combination with a therapeutically effective amount of a gram positive or gram negative antibiotic to prevent or treat secondary bacterial infections of the oral cavity or on the surface of dentures.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates the effect of α–MSH (1-13) and (11-13) and the peptide VPK-Ac-CC-Ac-KPV on *C. albicans* colony forming units compared to controls. All three molecules significantly decreased *C. albicans* colony forming units over a broad range of peptide concentrations.

Figure 2 represents a comparison of candidacidal activity of certain melanocortin peptides and fluconazole (all 10^{-6}M). The most effective of the melanocortin peptides were those including the C-terminal amino acid sequence of α–MSH, namely, α–MSH (1-13), (6-13) and (11-13).

Figure 3A shows untreated germination of *C. albicans*, i.e. blastopores.

Figure 3B shows a horse serum-induced germination of *C. albicans*.

Figure 3C shows the effect of α–MSH (1-13) treatment on germination of *C. albicans*

Figure 3D shows the effect of α–MSH (11-13) treatment on germination of *C. albicans*

Figure 4 illustrates the effect of α–MSH (1-13) and (11-13) on *C. albicans* killing by human neutrophils. Values are expressed as percent increase in killing vs. medium along. Scores are means ± SEM.

Figure 5 illustrates the effect of α–MSH (1-13), (11-13), and forskolin on cAMP content of *C. albicans*.

Figure 6 illustrates the inhibitory effect of α–MSH (1-13), (11-13), and forskolin on *C. albicans* colony forming units.

DETAILED DESCRIPTION OF THE INVENTION

α-MSH is a 13 amino acid, fungicidal peptide with the primary sequence SYSMEHFRWGKPV. In addition to its fungicidal properties it also anti-pyretic and anti-inflammatory. The C-terminal trimer, KPV, appears responsible for these properties. Lipton, J.M., Antipyretic and Anti-inflammatory Lys-Pro-Val- Compositions and Methods of Use, U.S. Patent No. 5,028,592, issued July 2, 1991; Lipton, J.M., Antipyretic and Anti-inflammatory Lys-Pro-Val- Compositions and Methods of Use, U.S. Patent No. 5,157,023, issued October 20, 1992; Catania, A., Lipton J.M., α-Melanocyte Stimulating Hormone in the Modulation of Host Reactions, *Endocr. Rev.* 14, 564-576 (1993); Lipton, J. M., Catania, A., Anti-inflammatory Influence of the Neuroimmunomodulator α–MSH, *Immunol. Today* 18, 140-145 (1997). herein incorporated by reference. All references are hearby incorporated by reference in their entirety. The core α–MSH sequence (4-10) has learning, memory and behavioral effects but little anti-pyretic and anti-inflammatory activity. Lipton, J.M., Catania, A., Anti-inflammatory Influence of the Neuroimmunomodulator α–MSH, *Immunol. Today* 18, 140-145 (1997). α–MSH, the α–MSH core and its tripeptide C-terminal have very low toxicity. Lipton, J.M., Catania, A., Anti-inflammatory Influence of the Neuroimmunomodulator α–MSH, *Immunol. Today* **18**, 140-145 (1997).

α-MSH is produced by the post translational processing of propriomelanocortin and shares the 1-13 primary sequence with adrenocortitrophic hormone (ACTH). Eberle, A. N., The Melanotropins, Karger, Basel, Switzerland (1988). It is secreted by a wide variety of cell types, including pituitary cells, monocytes, melanocytes, keratinocytes, epidermal cells and the epithelial cells of mucous membranes. Lipton, J.M., Catania, A., Anti-inflammatory Influence of

the Neuroimmunomodulator α–MSH, *Immunol. Today* **18**, 140-145 (1997); see also Catania et al., unpublished.

α-MSH reduces inflammation and fever by modulating the inflammatory cascade locally and systemically. Rajora, N., Ceriani, G., Catania, A., Star, R.A., Murphy, M.T., Lipton, J.M., α–MSH Production, Receptors and Influence of Neopterin, in a Human Monocyte/macrophage Cell Line, *H. Leukoc. Biol.* 59, 248-253 (1996); Star, R.A., Rajora, N. Huang, J., Stock, R.C., Catania, A., Lipton, J.M., Evidence of Autocrine Modulation of Macrophage Nitric Oxide Synthase by α–MSH, *Proc. Natl. Acad. Sci.* **92**, 8016-8020 (1995); Lipton, J.M., Ceriani, G., Macaluso, A., McCoy, D., Carnes, K., Biltz, J., Catania, A., Anti-inflammatory Effects of the Neuropeptide α–MSH in Acute, Chronic and Systemic Inflammation, *Ann. N.Y. Acad. Sci.* **741**, 137-148 (1994); Rajora, N., Boccoli, G., Burns, D., Sharma, S., Catania, A., Lipton, J.M., α–MSH Modulates Local Circulating Tumor Necrosis Factor A in Experimental Brain Inflammation, *J. Neurosci*, 17, 2181-2186 (1997); Richards, D.B., Lipton, J.M. Effect of α–MSH (11-13)(Lys-Pro-Val)on Fever in Rabbits, *Peptides* **5**, 815-817 (1984); Hiltz, M.E., Lipton, J.M., Anti-inflammatory Activity of a COOH-terminal Fragment of the Neuropeptide α–MSH, *FASEB J.* **3**, 2282-2284 (1989).

The broadest aspect of the invention is a composition and method of treatment of fungal pathologies of the oral cavity or fungal growth on the surface of dentures. In a preferred embodiment of the invention a therapeutically effective amount of one or more peptides selected from the group of peptides with a C-terminal sequence consisting of KPV, HFRWGKPV, and SYSMEHFRWGKPV is incorporated into a carrier. More preferably still, each peptide has the primary sequence of KPV or VPK-Ac-CC-Ac-KPV (Ac=Acetyl group). Pharmacologically effective concentrations may be as low as 10^{-12}M but may be as high 10^{-4} M. A preferred

embodiment of the invention utilizes peptide concentrations of 10^{-12}M to 10^{-10} M. Pharmacologically effective concentrations of these peptides may be incorporated into commercial formulations of creams, gels, mouthwashes, toothpastes, tablets, or atomized sprays.

Formulations of creams and gels are well known in the art. HARRY'S COMSETICOLOGY (Chemical Publishing, 7th ed. 1982); REMINGTON'S PHARMACEUTICAL SCIENCES (Mack Publishing Co., 18th ed. 1990).

Set forth below are examples of various formulations of the invention. As used below the term "Active ingredient" refers to one or more peptides selected from the group of peptides with a C-terminal sequence consisting of KPV, HRFWGKPV and SYSMEHFRWGKPV. Preferably, the active ingredient is KPV or VPK-Ac-CC-Ac-KPV.

An exemplary formulation of a gel based on the invention comprises:

Propylene Glycol	10.0g
PEG-Glyceryl Cocoate	10.0g
di-α-Tocopherol	.02g
Ascorbyl Palmitate	.10g
Propyl Gallate	.002g
Citric Acid, annhydr	.01g
Isopropanol	50.0g
Hydroxypropyl Methyl Cellulose	3.00g
Water	100g
Active ingredient	.2-.2*10^{-8}mg

An exemplary formulation of a cream comprises:

Glycerol	5.0g
Na_2-EDTA	.03g
Glycerides	10.0g
Cetyl Alcohol	1.0g
Stearyl Alcohol	1.0g
Glycerol mono Stearate	4.0g
Cetereth	2.0g
di-α-tocopherol	.02g
Water	100.0g
Active Ingredient	$.1\text{-}.1*10^{-8}$mg

Formulations for toothpastes, and mouthwashes are well known in the art. U.S. Patent No. 4,719,100; U.S. Patent No 4,314,990 and U.S. Patent No 4, 151,271. An exemplary formulation of a toothpaste comprises:

Sorbitol (70% aq.)	52.0g
Sodium Saccharine	.3g
Trisodium Phosphate	1.1g
Precipitated Silica	20.0g
Glycerine	18.0g
Sodium Flouride	.3g
Water	3.0g
Sodium Alkyl Sulfate (28.8 aq.)	4.0g
Flavoring	1.0g
Active Ingredient	$.2\text{-}.2*10^{-8}$mg

An exemplary formulation of a mouthwash comprises:

Water	89.0g
1-methoxypropanol	7.0g
n-propanol	1.0g
Saccharine	.06g
Glycerol	1.3g
Flavoring	1.0g
VPK-Ac-CC-Ac-KPV dimer	$.1\text{-}.1*10^{-8}$mg

In another preferred embodiment of the invention these compositions are topically or systemically applied to treat a candida infection of the oral cavity. Topical administration may be made with manual application of creams or gels, gargling of mouthwash, brushing with toothpaste, chewing on tablets, holding lozenges in the mouth or with an atomized spray. Systemic administration may be made by ingestion of hard tablets, soft tablets or capsules. In yet another preferred embodiment of the invention these peptides are used in combination with a therapeutically effective amount of a fungicide selected from the group consisting of: itraconazole, econazole, ketoconazole, miconazole and fluconazole. In yet another preferred embodiment of the invention these peptides are used in combination with a therapeutically effective amount of a gram positive or gram negative antibiotics selected from the group consisting of: aminglycosides, amoxicillin, ampicillin, azithramycin, erythromycin, nafcillin, penecillin, quinupuristin dalfopristin and vancomycin.

The formulation of tablets are well known in the art. An exemplary formulation of a hard gelatinous tablet comprises:

Gelatine Bloom 30	70.0mg
Maltodextrin MD 05	108.0mg
di-α-tocopherol	2.0mg
Sodium ascorbate	10.0mg
Microcrystalline cellulose	48.0mg
Magneisum stearate	2.0 mg
Active Ingredient	$.2*10^{-4}$-$.2*10^{-11}$ mg

An exemplary formulation of a hard tablet comprises:

Annhydrous lactose	130.5mg
Microcrystalline cellulose	80.0mg
di-α-tocopherol	2.0mg
Sodium ascorbate	10.0mg
Polyvinylpyrrolidone K30	5.0mg
Magnesium stearate	2.0mg
Active Ingredient	$.2*10^{-4}$-$.2*10^{-11}$ mg

The following examples teach the utility of a-MSH as a fungicide in general and anti-candidal fungicide in particular. Methods in microbiology, molecular biology and biochemistry used but not explicitly described in this disclosure are amply described throughout the literature and well within the ability of one skilled in the art.

The peptides used in the following examples include: α-MSH (1-13) (SEQ. ID. NO. 4), (4-10) (SEQ. ID. 002), (6-13) (SEQ. ID. 003), and (11-13) (SEQ. ID. NO. 1), all of which were N-acetylated and C-amidated, and ACTH (1-39) and (18-39) (CLIP). These peptides were prepared by solid-phase peptide synthesis and purified by reversed phased high performance liquid chromatography. Some examples also include a dimer of the amino acid sequence KPV

(SEQ. ID. NO. 1), VPK-Ac-CC-Ac-KPV (SEQ. ID. NO. 8), which also was N-acetylated and C-amidated (KPV dimer). Dimers can be formed by adding cysteines at the N-termini of any of the above polypeptides and allowing the cysteines of two polypeptides to form a disulfide bond. Both homo-dimers and hetero-dimers can be formed using this method.

C. albicans (clinical isolate) was obtained from the collection of the Department of Microbiology, Ospedale Maggiore di Milano and maintained on Sabouraud's agar slants and periodically transferred to Sabouraud's agar plates and incubated for 48 hours at 28° C. To prepare a stationary growth phase yeast, a colony was taken from the agar plate and transferred into 30ml Sabouraud-dextrose broth and incubated for 72 hours at 32° C. Cells were centrifuged and suspended in Hank's balanced salt solution ("HBSS") to the desired concentration. Viability, determined by the exclusion of .01% methylene blue, remained >98%.

Statistical significance disclosed in the examples below was analyzed using one-way analysis of variance and the Student's t test. Probability values greater than 0.05 were considered significant.

EXAMPLE 1

The first example suggests that α–MSH (11-13), (6-13) and (1-13) exhibit similar anti-candidal properties as flucanazole over an exceedingly broad range of concentrations.

C. albicans ($1x10^6$/ml in HBSS) was incubated in the presence of absence or α–MSH (1-13) or (11-13) at concentrations in the range of 10^{-15} M to 10^{-6} M for 2 hours at 37° C. Cells were then washed in cold distilled waste and diluted with HBSS to a concentration of 100 organisms/ml. One-ml aliquots were dispensed on blood agar plates and incubated for 48 hours at 37° C. Organism viability was estimated from the number of colonies formed.

In subsequent experiments using familiar procedures we compared activity of α–MSH (4-10), (6-13), (11-13), ACTH (1-39), (18-39) and fluconazole, the latter an established antifungal agent. Melanocortin peptides and fluconazole were tested in concentrations of 10^{-6} M to 10^{-4} M. There were at least six replicates for each concentration of peptide.

Fig. 1 shows that *C. albicans* colony forming units (CFU) were greatly reduced by α–MSH (1-13) and (11-13). Fig. 1 also shows that the VPK-Ac-CC-Ac-KPV peptide also inhibited *C. albicans* colony formation). Concentrations of all three peptides from 10^{-12}M to 10^{-4}M had significant inhibitory effects on CFU ($p<.01$ vs. control).

Fig. 2 demonstrates that in experiments comparing the relative potency of 10^{-4}M melanocortin peptides in reducing *C. albicans* viability, α–MSH (11-13), (6-13) and (1-13) were the most effective. Their inhibitory activity was similar to that of equimolar fluconazole. The core α–MSH sequence (4-10), which has behavioral effects but little anti-inflammatory activity, caused approximately 50% inhibition of CFU. Fig. 2 also shows that although this inhibitory effect was substantial ($p<.01$ vs. control), it was significantly less than that caused by α–MSH fragments bearing the KPV signal sequence, i.e., α–MSH (6-13) and (11-13) ($p<.01$), or the parent molecule α–MSH (1-13)($p<.05$). ACTH (1-39) and the ACTH fragment (18-39) did not reduce *C. albicans* viability. Even higher concentrations of these ACTH peptides (up to 10^{-4}M) were likewise ineffective in reducing *C. albicans* CFU (results not shown in the figures).

These results show that α–MSH(1-13), its C-terminal tripeptide (11-13), and other α–MSH fragments have significant fungicidal effects against *C. albicans*. The most effective of the α–MSH peptides were those including the C-terminal amino acid sequence KPV of the α–MHS sequence, i.e., α–MSH (1-13), (6-13) and (11-13). In addition, the sequence VPK-Ac-CC-Ac-KPV has also been shown to be at least as effective α–MSH (11-13) against microbes.

The α–MSH cores sequence (4-10), which is known to influence learning and memory, but has little antipyretic and anti-inflammatory influence, was effective, but less so. The ACTH peptides (1-39) and (18-39) did not have significant candidacidal effects. These observations indicate that antifungal activity is not common to all melanocortin peptides, but rather that is specific to α–MSH amino acid sequences, and most particularly to the C-terminal amino-acid sequences of α–MSH. This strongly suggests that α–MSH(1-13), its C-terminal tripeptide (11-13), and other α–MSH fragments could server as a basis for a therapeutic treatment of acute and chronic candidal infections of the oral cavity or as antifungal agent against candidal growth on denture surfaces.

EXAMPLE 2

Example 2 demonstrates that α–MSH (1-13), (6-13) or (11-13) strongly inhibits Candidal germination. *C. albicans* from stationary phase cultures were washed twice with distilled water and suspended in HBSS to a final concentration of 2×10^6/ml. Hyphal growth was induced by addition of 10% inactivated horse serum (GIBCO/BRL, Great Britain) to yeast incubated for 45 minutes at 37° C with continuous shaking. Horse serum was removed by washing cells twice with HBSS and incubation was continued for 60 minutes at 37° C in the presence of α–MSH (1-13), (6-13) or (11-13) at a concentration of 10^{-6}M with continuous shaking. The percentage of filamentous cells was evaluated under a light microscope with the aid of hemocytometer. Experiments were run in triplicate and at least 200 cells were scored. Photomicrographs were taken with a MC100 camera attached to an Axioskop Zeiss microscope.

Figs. 3A–D show that coincubation of *C. albicans* with α–MSH (1-13) or (11-13) inhibited germ tube formation induced by horse serum, α–MSH (1-13) caused 28-32% reduction

in the number of filamentous cells; the tripeptide inhibited germination by 54-58%. The octapeptide α–MSH (6-13) had similar activity (approximately 50% inhibition)(not shown).

The pathogenesis of C. albicans infection involves adhesion of yeast cells to epithelial cells, commonly found in the mucosal membranes of the ears, eyes, nose and throat and/or endothelial cells, followed by morphologic switching of the yeast cells from the ellipsoid blastospore to various filamentous forms: germ tubes, pseudohyphae and hyphae. Gow, N.A., Germ Tube Growth of Candida Albicans, *Curr. Topics Med. Mycol.* **8**, 43 –45 (1997). The results also show that in addition to direct candicidal properties, α–MSH(1-13), its C-terminal tripeptide (11-13), and other α–MSH fragments interfere with germination and adhesion of candida to the epithelium. This suggests that if the germination and adhesion of candida could be interfered with, the invasive forms of chronic candidiasis that innervate the epithelium could be treated with therapy based upon α–MSH(1-13), its C-terminal tripeptide (11-13), and other α–MSH fragments.

EXAMPLE 3

Example 3 illustrates that α-MSH and its derivatives exhibit their anti-candidal properties without compromising the ability of human neutrophils to independently combat Candida. Venous blood (20ml) from health volunteers was anticoagulated with heparin. Neutrophils were isolated using dextran sedimentation and Ficoll-Hypaque (Sigma Chemical Co., St. Louis, Mi., USA) centrifugation. Erythrocytes were lysed via hypotonic shock. Neutrophils represented at least 97% of the cell suspension. Cell viability, estimated by trypan blue exclusion, was >98%. Neutrophils were suspended to a final concentration in HBSS.

C. albicans (1×10^6) were opsonized with human AB serum in a shaking water bath for 30 minutes at 37° C. Organisms were then incubated with neutrophils in medium or in medium with α–MSH (1-13) or α–MSH (11-13) in concentrations of 10^{-15} M to 10^{-4} M in a shaking water bath for 2 hours at 37° C. After incubation, the culture tubes were placed on ice to stop growth and extracellular organisms were washed twice with centrifugation at 1000 x g at 4° C. A 2.5% sodium desoxycholoate solution was added to obtain a suspension of 10^6 cells/ml. Two 1/100 serial dilutions in HBSS were made to obtain a final suspension of 100 cells/ml. Aliquots of 1 ml were dispensed on blood agar plates and incubated for 48 hours at 37° C. Colony forming units (CFUs) were counted at the end of the incubation period. Experiments were run in triplicate and repeated using blood from 5 different donors.

Fig. 4 shows that α–MSH(1-13) and (11-13) enhanced the killing of *C. albicans* by human neutrophils when administered in concentrations of 10^{-12}M to 10^{-4}M ($p<.01$). Therefore, enhanced killing occurred over a very broad range of concentrations including picomolar concentrations, i.e. the quantity of α–MSH found in human placenta. Catania, A., Airaghi, L., Garofalo, L., Cutuli, M., Lipton, J.M., The Neuropeptide α–MSH in AIDS and Other Conditions in Humans, *Ann. N.Y. Acad. Sci.* **840**, 848-856 (1998).

Reduced killing of pathogens is a dire consequence of therapy with corticosteroids and nonsteroidal anti-inflammatory drugs during infection. Stevens, D.L., Could Nonsteroidal Anti-inflammatory Drugs (NSAIDS) Enhance Progression of Bacterial Infections to Toxic Shock Syndrome?, *Clin. Infect. Dis.*, **21**, 977-80 (1997); Capsoni, F., Meroni, P.L., Zocchi, M.R., Plebani, A.M., Vezio, M., Effect of Corticosteroids on Neutrophil Function: Inhibition of Antibody-dependent Cell-mediated Cytotoxicity (ADCC), *J. Immunolpharmacol.* **5**, 217-230 (1983). This effect is particularly dangerous in immunocompromised patients.

These results also suggest that α–MSH(1-13), its C-terminal tripeptide (11-13), and other α–MSH fragments would be useful for treatment of candidiasis in immunocompromised patients since these peptides appear not to reduce neutrophil chemotaxis and thus would not further comprise the immune system.

EXAMPLE 4

Example 4 suggests a cellular mechanism to explain how α-MSH exerts its anti-candidal properties. *C. albicans* (10^6/ml), permeabilized with toluene/ethanol, were incubated at 37° C with continuous shaking in the presence of 10^{-6} M α–MSH (1-13), (11-13), forskolin, an agent known to increase intracellular cAMP, or in medium alone. The reaction was stopped after 3 minutes by the addition of ice cold ethanol, cAMP was measured in duplicate using a commercial enzyme immunoassay (EIA) kit (Amersham, United Kingdom) after extraction via the liquid-phase method according to manufacturer's instructions. The effect of forskolin (10^{-6} M) on *C. albicans* colony formation was determined using the same procedure as for α–MSH peptides.

Because many of the effects of α–MSH are known to be mediated by induction of cAMP, we measured effects of α–MSH peptides on cAMP accumulation in *C. albicans*. Fig. 5 shows that α–MSH (1-13) and (11-13) enhanced cAMP content in the yeast. Fig. 6 shows the increase was of the same order of magnitude as that induced by equimolar forskolin, an adenylate cyclase activator. To determine whether increases in cAMP could be responsible for reduction in CFU, we tested the effects of forskolin on *C. albicans* viability. Results showed that 10^{-6}M forskolin markedly inhibited *C. albicans* CFU relative to control ($p<.01$). Fig. 6 demonstrates that the inhibitory effect was similar to that exerted by α–MSH.

The mechanism of action of natural antimicrobial agents is only partly understood. Most of these peptides, including the defensins, alter membrane permeability and impair internal homeostasis of the organism. The first contact is made between the cationic groups of the peptide and the negatively charged head of the target membrane. Then, the tertiary structure determines the mode of insertion of the peptide into membranes where they form ion channels or pores that disrupt cell integrity. It is known that cAMP-enhancing agents inhibit mRNA and protein synthesis in *C. albicans*. Bhattacharya, A., Datta, A., Effect of Cyclic AMP on RNA and Protein Synthesis in *C. albicans*, *Biochem. Biophys. Res. Commun.* **77**: 1483-44 (1977).

In the present experiments it is shown that α–MSH induces cAMP accumulation in *C. albicans* and also that the cAMP-inducing agent forskolin inhibited colony formation. Without being limited by this theoretical explanation, it may be that the antimicrobial effect was caused by enhancement of this mediator.

EXAMPLE 5

Example 5 suggests fucntional equivalents to α-MSH and its derivatives. As used herein, a biological functional equivalent is defined as an amino acid sequence that is functionally equivalent in terms of biological activity.

Although the specific amino acid sequences described here are effective, it is clear to those familiar with the art that amino acids can be substituted in the amino acid sequence or deleted without altering the effectiveness of the peptides. Further, it is known that stabilization of a the α–MSH sequence can greatly increase the activity of the peptide and that substitution of amino acid D-forms for L-forms can improve or decrease the effectiveness of peptides. For example, a stable analog of α–MSH, [Nle^4,D-Phe^7]-α–MSH which known to have marked

biological activity on melanocytes and melanoma cells, is approximately 10 times more potent than the parent peptide in reducing fever. Holdeman, M., and Lipton, J.M., Antipyretic Activity of a Potent α–MSH Analog, *Peptides* 6, 273-5 (1985). Further, adding amino acids to the C-terminal α–MSH (11-13) sequence can reduce or enhance antipyretic potency. Deeter, L.B., Martin, L.W., Lipton, J.M., Antipyretic Properties of Centrally Administered α–MSH Fragments in the Rabbit, *Peptides* 9, 1285-8 (1989). Addition of glycine to form the 10-13 sequence slightly decreased the potency; the 9-13 sequence was almost devoid of activity, whereas the potency of the 8-13 sequence was greater than that of the 11-13 sequence. It is known that Ac-[D-K^{11}]-α–MSH 11-13-NH_2 has the same general potency as the L-form of the tripeptide α–MSH 11-13. Hiltz, M.E., Catania, A., Lipton, J.M., Anti-inflammatory Activity of α–MSH (11-13) Analogs; Influences of Alterations in Stereochemistry, *Peptides* 12, 767-71 (1991). However, substitution with D-proline in position 12 of the tripeptide rendered it inactive. Substitution with the D-form of valine position 13 or with the D-form of lysine at position 11 plus the D-form of valine at position 13 resulted in greater anti-inflammatory activity than with the L-form tripeptide. These examples indicate that alterations in the amino acid characteristics of the peptides can influence activity of the peptides or have little effect, depending upon the nature of the manipulation.

It is also believed that biological functional equivalents may be obtained by substitution of amino acids having similar hydropathic values. Thus, for example, isoleucine and leucine, which have a hydropathic index +4.5 and +3.8 , respectively, can be substituted for valine, which has a hydropathic index of +4.2, and still obtain a protein having like biological activity. Alternatively, at the other end of the scale, lysine (-3.9) can be substituted for arginine (-4.5), and so on. In general, it is believed that amino acids can be successfully substituted where such

amino acid has a hydropathic score of within about +/- 1 hydropathic index units of the replaced amino acid.

EXAMPLE 6

An elderly diabetic patient presents with white plaque like lesions on the tongue. The patient has been a diabetic since childhood with a poor history of blood sugar control. Samples of the white keratinized lesions show moderate candidal fungal hyphae innervation. The patient is prescribed a pharmacologically effective amount of amphotericin and a pharmacologically effective concentration of α-MSH.

Examples 1-6 demonstrate the anti-candidal properties and uses of α-MSH and/or its derivatives. These are only illustrative and are not intended to limit the invention. It is understood that modifying the examples above does not depart from the spirit of the invention. It is further understood that the examples can be applied independently or in combination with each other.